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Use of PGMY Primers in L1 Consensus PCR Improves Detection of Human Papillomavirus DNA in Genital Samples

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The novel PGMY L1 consensus primer pair is more sensitive than the MY09 and MY11 primer mix for detection and typing with PCR of human papillomavirus (HPV) DNA in genital specimens. We assessed the diagnostic yield of PGMY primers for the detection and typing of HPV by comparing the results obtained with PGMY09/PGMY11 and MY09/MY11/HMB01 on 299 genital samples. Amplicons generated with PGMY primers were typed with the line blot assay (PGMY-line blot), while HPV amplicons obtained with the degenerate primer pool MY09/MY11/HMB01 were detected with type-specific radiolabeled probes in a dot blot assay (standard consensus PCR test). Cervicovaginal lavage samples ($N = 272$) and cervical scrape samples ($N = 27$) were tested in parallel with both PCR tests. The PGMY-line blot test detected the presence of HPV DNA more frequently than the standard consensus PCR assay. The concordance for HPV typing between the two assays was 84.3% (214 of 255 samples), for a good kappa value of 0.69. Of the 177 samples containing HPV DNA by at least one method, 40 samples contained at least one HPV type detected only with PGMY-line blot, whereas positivity exclusively with the standard consensus PCR test was found for only 7 samples ($P < 0.001$). HPV types 45 and 52 were especially more frequently detected with PGMY than MY primers. However, most HPV types were better amplified with PGMY primers, including HPV-16. Samples with discordant results between the two PCR assays more frequently contained multiple HPV types. Studies using PGMY instead of MY primers have the potential to report higher detection rates of HPV infection not only for newer HPV types but also for well-known genital types.

Human papillomavirus (HPV) infection is a very strong and independent predictor of the presence of squamous intraepithelial lesions and invasive cancer of the uterine cervix (14, 30, 34). Most HPV infections in women are transient and only a minority of women infected with HPV develop persistent infection that may evolve into squamous intraepithelial lesions (10, 13, 21, 27). The 40 HPV genotypes that infect the anogenital tract of men and women are classified into low-risk and high-risk categories based on their association with malignant lesions and phylogenetic relationships (9, 14, 30, 35, 36).

The modest sensitivity level of HPV detection methods used in initial studies on the natural history and determinants of HPV infection resulted in misclassification of HPV infection status. As a consequence of misclassification of individuals, conflicting results from various studies have been reported. This problem was resolved in the 1990s by using nucleic acid amplification assays, mainly PCR (6, 12). Because of the genetic diversity of genital HPVs, the use of type-specific PCR assays is impractical for epidemiological studies for which accurate HPV typing is essential (1). Consensus PCR assays have been devised to amplify most relevant genital types in one reaction and also detect novel HPV genotypes.

Three assays target conserved sequences in the HPV L1 gene. The MY09/MY11/HMB01, GP5+/GP6+, and SPF1/SPF2 consensus primer sets can amplify a wide spectrum of genital HPV types and have been used in several large-scale epidemiological studies (3, 9, 20, 23–25). The MY09/MY11/HMB01 PCR assay has been widely used and adapted recently with success to a reverse nonisotopic detection of HPV amplified DNA, the line blot assay (16). For HPV typing, the line blot assay compared favorably to a dot blot assay using type-specific radiolabeled oligonucleotide probes (4).

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The degenerate pool of primers MY09/MY11/HMB01 amplifies a broad spectrum of HPV genotypes with various levels of sensitivity (15, 19, 28, 33). The insertion of nucleotide bases at positions of degeneracy is a random and irreproducible process. Lot-to-lot variations among separate syntheses of MY09/MY11/HMB01 primers could result in differences in type-specific amplification efficiencies. Synthesis of degenerate primers does not ensure an equivalent representation of all degenerate primers (15).

The new PGMY09/PGMY11 set of consensus primers was designed to eliminate the degeneracies and to improve the sensitivity, specificity, and reproducibility of L1 consensus PCR (15). In one study, PGMY primers improved the sensitivity of amplification of genital HPV types over the MY primer pair both in vitro and in clinical specimens (15).

We report here the results achieved on clinical specimens with the PGMY09/PGMY11 primer set using the line blot assay (PGMY-line blot) (16) compared with those found by MY09/MY11/HMB01 primers using radiolabeled type-specific probes in a dot blot assay (standard consensus PCR test). This comparison allowed us to identify the types for which the new primer pair was more efficient. We also investigated the ability of PGMY primers to detect HPV types in samples with multiple HPV genotype infections.

MATERIALS AND METHODS

Cell lines and clinical specimens. The cervical carcinoma cell line HeLa (which contains 40 copies of HPV-18 DNA per cell) was obtained from the American Type Culture Collection (Rockville, Md.). Two hundred ninety-nine genital specimens were collected from 228 women enrolled in two cohort studies that investigated the determinants of persistent HPV infection. Two hundred seventy-two cervicovaginal lavage samples were from the Canadian Women's HIV Study (5, 18, 26). This study evaluates the relationship of genital HPV infection and persistence to cervical disease progression in relation to human immunodeficiency virus (HIV)-induced immune deficiency.

One hundred fifty-seven cervicovaginal lavage samples were consecutive samples obtained from 157 women, and 115 cervicovaginal lavage samples were selected on the basis of initial results obtained with MY09/MY11/HMB01 amplification reactions in previous work (4) (29 samples contained one HPV genotype, 30 samples contained more than one genotype, 29 samples had generated discordant results between different PCR assays, and 27 samples were HPV negative). This selection ensured the inclusion of all HPV types detected in the standard consensus PCR test as well as the inclusion of specimens containing multiple HPV types. Twenty-seven consecutive cervical brushing samples were from an ongoing study on the determinants of HPV persistence in young adult women attending McGill University (29). Consent was obtained from each participant. Both projects had the approval of the ethics committees of the institutions involved.

Processing of clinical samples. Cervicovaginal lavage samples were centrifuged at 2,500 rpm for 10 min at 4°C, resuspended in 500 μl of 10 mM Tris-HCl (pH 8.2), and stored frozen at −70°C until processed (5). Cell suspensions were thawed, lysed by addition of Tween 20 at a final concentration of 0.8% (vol/vol), and digested with 250 μg of proteinase K per ml for 2 h at 45°C. Cell lysates were boiled for 10 min and stored at −70°C until tested.

Exfoliated endo- and ectocervical cells from the uterine cervix were obtained with the Accelon Combi cervical biosampler and resuspended in 2 ml of 10 mM Tris-HCl (pH 7.4) with 0.1 mM EDTA (TE) buffer. Two hundred microliters of cell suspension was lysed with Tween 20 (final concentration of 0.8% [vol/vol]) and digested with 250 μg of proteinase K per ml at 45°C for 2 h. Lysates were purified with GlassMAX resin (Gibco-BRL, Burlington, Canada) according to the recommendations of the manufacturer, and resuspended in 50 μl of TE. Cell lysates were boiled for 10 min and stored at −70°C until tested. Five microliters of processed sample was tested in each PCR assay. All samples yielded a β-globin amplimer with the PC04/GH20 primers, confirming the presence of amplifiable DNA (3, 5).

Standard consensus PCR test. All samples were tested with two PCR assays as described below without knowledge of previous results and clinical status. HPV DNA was amplified under standard conditions with the L1 consensus HPV primers MY09/MY11 and primer HMB01, as previously described (5, 8, 20). The amplification mixture contained 6.5 mM $MgCl_2$, 50 mM KCl, 2.5 U of Ampli *Taq* DNA polymerase (Roche Molecular Diagnostics, Mississauga, Canada), 200 μM (each) dATP, dCTP, dGTP, and dTTP, and 50 pmol of each primer pool. Negative and weak positive (25 HPV-18 DNA copies) controls were included to monitor contamination and overall endpoint sensitivity of each PCR run. Amplifications were performed in a 9600 Thermocycler (Perkin-Elmer Cetus, Montréal, Canada) for 40 cycles with the following cycling parameters: 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min.

Amplified products were spotted onto nylon membranes and were hybridized first with an HPV generic probe mixture under low-stringency conditions. The generic probe mixture was generated by amplification in separate reactions of HPV-16, HPV-18, and HPV-31 plasmids with type-specific nested primers and ^{32}P-labeled deoxynucleotides (17). Amplified nested L1 amplicons were mixed and used as a generic probe that efficiently detects common genital types (5, 17). Membranes were then washed in boiling water and reacted under stringent conditions with ^{32}P-labeled oligonucleotide probes for types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, and 58 (3, 6, 20). We have described elsewhere the measures to avoid false-positive reactions due to contamination (5).

PGMY-line blot assay. Lysates were all amplified with the consensus L1 PCR protocol as previously described for the PGMY09/PGMY11 primer set (15). The amplification step of this assay differs from the standard consensus PCR test in several aspects: by the use of 4 mM $MgCl_2$, 7.5 U of Ampli *Taq* Gold DNA polymerase (Perkin Elmer Cetus), 600 μM dUTP, and biotinylated PGMY primers instead of MY primers. Sequences of these primers were recently published (15). The ultrasensitive amplification profile was used in a TC 9600 thermal cycler: activation of AmpliTaq Gold at 95°C for 9 min; 95°C denaturation for 1 min, 55°C annealing for 1 min, and 72°C extension for 1 min for 40 cycles, and then 5-min terminal extension at 72°C. Detection of biotinylated amplicons with the line blot assay was completed as described previously (16). The probe mixes for the following 27 HPV genotypes had been fixed on distinct lines on each strip: types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 82, 83, 84, and MM9, and were provided by Roche Molecular Systems.

When discordant results between the standard consensus PCR and the PGMY-line blot tests were encountered, samples were retested with both assays. PCR products from amplification with PGMY primers were also spotted onto a nylon filter and hybridized with type-specific radiolabeled oligonucleotide probes as in the standard consensus PCR assay. These lysates were also tested with biotinylated MY09/MY11/HMB01 primers and the line blot as previously described (4, 16).

Statistical methods. The crude percent agreement between the two detection methods was the percentage of samples with identical results by both methods. Agreement for overall positivity (HPV DNA positive), for positivity for high-risk HPV types as a group (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58), and for positivity for each type was calculated. The unweighted kappa statistic was calculated to adjust for chance agreement between HPV detection methods (11). In general, a kappa value above 0.75 indicates excellent agreement, between 0.40 and 0.75 indicates fair to good agreement, and below 0.40 represents poor agreement beyond chance. The mean number of types detected per sample by each PCR test was compared using the Mann-Whitney rank sum test, since the spectrum of types per sample was not normally distributed. The two-sided McNemar's chi-square analysis for matched-pair data was performed to analyze contingency tables comparing both PCR tests. Proportions were compared with the *z* statistic test.

RESULTS

Coamplification of β-globin and HPV using consensus L1 primers can reduce the level of sensitivity for HPV detection (4). To determine if we could use β-globin and HPV coamplification in the PGMY-line blot assay, 17 HPV-positive samples, for which we had obtained different results with and without coamplification in previous work (4), were tested with PGMY-line blot with and without β-globin primers. In 15 of these 17 samples, at least one HPV type (range, 1 to 3; median, 1; mean ± standard deviation, 1.3 ± 0.6) was not detected when coamplification was used (data not shown). All 15 samples contained more than one HPV type by the PGMY-line

TABLE 1. Comparison of PGMY-line blot assay and the standard consensus PCR test for detection of 14 genotypes of HPV DNA in 299 genital samples[a]

PGMY-line blot result	Standard consensus PCR test result for no. (%) of samples		
	Positive	Negative	Total
Positive	157 (52)	19 (6)	176
Negative	1 (1)	122 (41)	123
Total	158	141	299

[a] The agreement between the two PCR assays for the presence of HPV DNA was 93.3%, for a kappa value of 0.87.

blot assay. We thus avoided HPV and β-globin coamplification in both PCR assays.

The standard consensus PCR test and the PGMY-line blot assay were compared on a total of 299 specimens. HPV DNA was detected in 203 (67.9%) and 184 (61.5%) samples with the PGMY-line blot assay and the standard consensus PCR test, respectively. Both assays detected the presence of HPV DNA in 178 samples and were negative in 90 samples, while 25 samples were positive only with the PGMY-line blot and 6 samples were positive only with the standard consensus PCR test. A very good agreement of 89.6% (268 of 299 samples) was found between the two PCR methods for the detection of HPV DNA (kappa value of 0.77, $P = 0.001$).

The generic probe in the standard consensus PCR test identified the presence of HPV DNA in 179 (59.9%) of 299 samples. However, it failed to detect the presence of at least one HPV type per sample in 30 samples, including types 42 (in 7 samples), 66 (in 5 samples), 54 (in 4 samples), 84, 52, 53, and 55 (in 3 samples each), 83, 56, 16, 18, and 35 (in 2 samples each), and 68 (in 1 sample), that were identified by PGMY-line blot. The generic probe identified HPV DNA sequences in 26 samples that did not react with the type-specific probes of the standard consensus PCR test. Fourteen of these samples contained HPV types not included in the panel of 14 probes used in the standard consensus PCR test, and 5 contained HPV DNA that could not be typed by either assay. The remaining seven samples contained HPV DNA sequences from one of the 14 types detected with the type-specific probe used in the standard consensus PCR test.

The following analyses were restricted to the 14 genotypes (types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, and 58) detectable by the standard consensus PCR test and the PGMY-line blot assay. Results from the generic probe in the standard consensus PCR tests were not considered for these comparisons. Specimens that tested positive by the PGMY-line blot assay for an HPV type not included in the latter 14 types were considered negative. First, the PGMY-line blot assay and the standard consensus PCR test were compared for their ability to detect the presence of HPV DNA in 299 genital specimens (Table 1). HPV DNA was detected in 158 (52.8%) and 176 (58.9%) of the 299 samples by the standard consensus PCR and the PGMY-line blot assays, respectively. There was an excellent agreement of 93.3% between the PCR assays for the presence of HPV DNA (kappa value = 0.87; McNemar's chi-square, $P < 0.001$). Of the 158 samples classified as positive for HPV DNA by the standard consensus PCR test, 157 were positive by PGMY-line blot (sensitivity of 99.4%).

TABLE 2. Detection of HPV types by PGMY-line blot assay and the standard consensus PCR test among 177 HPV-positive genital samples

HPV type	No. of samples positive[a]			
	Both PCR assays	Standard PCR only	Line blot only	Total
6	20	0	3	23
11	4	0	1	5
16	26	0	6	32
18	20	0	4	24
26	nd	nd	3	3
31	17	1	1	19
33	15	1	2	18
35	16	0	3	19
39	19	0	2	21
40	nd	nd	3	3
42	nd	nd	33	33
45	8	0	4	12
51	27	0	4	31
52	27	0	9	36
53	34	0	6	40
54	nd	nd	26	26
55	nd	nd	33	33
56	29	4	3	36
57	nd	nd	0	0
58	24	1	1	26
59	nd	nd	6	6
66	nd	nd	23	23
68	nd	nd	24	24
82	nd	nd	1	1
83	nd	nd	25	25
84	nd	nd	30	30
MM9	nd	nd	10	10

[a] nd, not determined.

We then determined if PGMY-line blot could improve the type-specific sensitivity of L1 consensus PCR. The distribution of the 27 types detected by the PGMY-line blot and the 14 genotypes detected by the standard consensus PCR assay in 177 HPV-positive samples by one or both assays is presented in Table 2. Considering typing results only for the 14 types detected with both assays (Table 3), there was a good agreement beyond chance of 84.3% between the two PCR assays (kappa = 0.69; $P < 0.001$, McNemar's chi-square). The PGMY-line blot detected HPV types undetected by the standard consensus PCR test in 40 (13.4%) of 299 samples, and the standard consensus PCR detected types undetected by the PGMY-line blot in 7 (2.3%) samples.

In seven samples, the standard consensus PCR assay identified the presence of one HPV type (HPV type 56 in 4 sam-

TABLE 3. Comparison of PGMY-line blot assay and the standard consensus PCR test for HPV DNA genotyping results for 14 types in 299 genital samples[a]

PGMY-line blot result	Standard consensus PCR test result for no. (%) of samples		
	Positive	Negative	Total
Positive	130 (44)	40 (13)	170
Negative	7 (2)	122 (41)	129
Total	137	162	299

[a] There was an 84.3% agreement between the two PCR assays (kappa = 0.69).

ples, types 58, 31, and 33 in 1 sample each) that was not detected with PGMY-line blot. In all cases, the signals obtained in the dot blot assay were weak. There was a significantly greater proportion of multiple HPV type infections in these discordant samples (6 [85.7%] of 7 samples) than in concordant HPV-positive samples (53 [40.8%] of 130 samples, $P = 0.05$). PCR products from amplification with PGMY primers of these seven samples were spotted on a filter and hybridized with type-specific isotopic probes used in the standard consensus PCR test. None of the amplicons tested contained the HPV types undetected with the PGMY-line blot, suggesting that amplification had not occurred with PGMY primers. The relative sensitivity for HPV type detection of PGMY09/PGMY11 reached 95.6% (151 of 158 MY09/MY11/HMB01-positive samples). On retesting with PGMY-line blot, all seven samples turned positive for the type undetected in the first run. They were thus considered true false-negative results of PGMY-line blot.

In 40 discordant samples, one to three HPV types per sample detected with PGMY-line blot were undetected with the standard consensus PCR assay. Nineteen (47.5%) of these 40 samples did not contain HPV DNA sequences by the standard consensus PCR test. As shown in Table 2, all 14 HPV genotypes were more likely to be detected by PGMY-line blot than by the standard consensus PCR assay. Considering the 14 HPV genotypes detectable with both PCR assays, samples positive only with PGMY-line blot were compared to samples positive with both assays (Table 2) to assess if some genotypes were more easily detected than others with PGMY-line blot. There was a statistically significant difference by HPV genotype in the number of samples positive only with the PGMY-line blot ($P = 0.01$, Pearson chi-square test).

To determine which HPV genotype(s) accounted the most for this effect, the contingency table was further subdivided arbitrarily into three categories depending on the proportion of total positive samples (last column of Table 2) reacting only with PGMY-line blot: types 31, 39, 56, and 58 were combined (between 1.0 and 10.0% of HPV-positive samples reacting only with PGMY-line blot), types 6, 11, 16, 18, 33, 35, 51, and 53 were combined (10.1 to 20.0%), and types 45 and 52 were combined (>20%). We did not find a significant difference in the number of samples containing HPV types detected only with PGMY-line when HPV genotypes within each group were compared ($P = 1.00$, 1.00, and 0.851, respectively). When groups were compared two by two, a significant difference was found in the number of samples positive only by PGMY-line blot when categories 31/39/56/58 and 45/52 were compared after the Bonferroni correction for a total of three comparisons ($P = 0.036$). The comparisons of groups 6/11/16/18/33/35/51/53 with 45/52 and 6/11/16/18/33/35/51/53 with 31/39/56/58 did not reach statistical significance ($P = 0.224$ and 0.164, respectively).

The latter 40 discordant samples had a greater proportion of multiple type infections (26 [65.0%] of 40 HPV-positive samples) than HPV-positive samples with concordant results (52 [40.0%] of 130 HPV-positive samples, $P = 0.01$). These 40 samples also contained a greater number of types per sample than HPV-positive samples with concordant results ($P = 0.07$, Mann-Whitney rank-sum test). Of these 40 discordant samples, 2 could not be tested further because of limited amounts of lysates, 4 samples were considered to have generated initial false-positive PGMY-line blot results because initial results were not reproduced on repeat testing (once each HPV types 6, 45, 52, and 56), and 34 samples generated the same results when retested with PGMY-line blot.

The 34 latter samples were retested with the standard consensus PCR test using MY09/MY11/HMB01 from a different oligonucleotide synthesis lot and with the line blot test using biotin-labeled MY09/MY11/HMB01 as described previously (4). Twenty-five (65.8%) samples tested positive in at least one of these tests for the HPV type that had been detected initially with PGMY-line blot, although this was inconsistent between runs (data not shown). These samples were thus false-negatives of the standard consensus PCR test and true-positives of the PGMY-line blot. The hybridization of biotin-labeled PCR products generated by PGMY primers from these 25 samples using radiolabeled probes confirmed the presence of amplicons from the HPV type identified with PGMY- line blot. For the other nine samples containing HPV DNA sequences from HPV types 52 (three samples), 35 (three samples), 53 (two samples), and 6, 16, 51, and 56 (one sample each), repeat testing did not solve the discrepancies.

For all nine discordant samples, radiolabeled probes confirmed the presence of type-specific amplicons generated by PGMY primers. Amplification of discordant samples with GP5+/GP6+ primers and detection with type-specific probes confirmed the presence of the types detected only with PGMY-line blot in six of nine samples (8, 22, 28).

More than one HPV type per sample was detected in 69 (43.7%) of 158 HPV-positive samples using the standard consensus PCR test and in 81 (46.0%) of 176 HPV-positive samples using the PGMY-line blot ($P = 0.755$). In 65 samples both assays identified multiple HPV types, and in 77 samples both detected only one HPV type. In 15 samples, the PGMY-line blot identified more than one HPV type while the standard consensus PCR detected only one, and the opposite occurred in 4 samples ($P = 0.022$).

Since each of the 299 samples was tested for the presence of 14 HPV types, 4,186 results for HPV typing could be compared between the two PCR assays. By considering each type individually, 4,131 of 4,186 results (98.7%) were concordant between these two assays. When concordance was calculated after exclusion of HPV-negative samples by both assays, 2,422 (97.7%) of 2,478 HPV-positive results were identical in both assays.

DISCUSSION

We compared two primer systems for PCR amplification of HPV DNA in genital specimens obtained from women from two different clinical populations. Previous work had shown an increased sensitivity of PGMY primers over MY09/MY11/HMB01 degenerate primers for the detection and typing of HPV DNA in clinical specimens when amplicons were tested with the line blot assay (15). We carried out the present study to assess the relative gain in diagnostic yield of using the PGMY-line blot assay instead of a conventional PCR test using MY09/MY11/HMB01 primers and radiolabeled probes to detect PCR products. Our results indicate that HPV detection and typing can be improved to various degrees for most genital

types. The increased sensitivity of PGMY-line blot was greater for HPV typing results than for identification of HPV DNA-positive samples.

The 90% to 93% agreement between the PGMY and MY systems, whether considering all results or those for 14 genotypes, is similar to the levels of agreement reported in the first evaluation of PGMY primers and in other studies comparing two consensus L1 PCR systems (15, 19, 28, 31). The generic probe in the standard consensus PCR test failed to detect the presence of HPV DNA, especially from types 42, 66, and 54. This underscores the importance of using a generic probe that reacts with the broadest possible spectrum of HPV sequences. Nevertheless, the generic probe identified HPV DNA sequences that were not typed by the standard consensus PCR test in 14 samples or were not typed by either PCR test in 5 samples. This indicates the need to design tests that will screen the greatest number of HPV types. The line blot assay is an important step towards this goal.

For a limited number of samples, results generated by PGMY-line blot could not be reproduced on repeat testing. This could represent contamination of the first PCR run, although our negative controls always scored negative, or variable amplification of very small amounts of target DNA. In seven samples, an HPV type was detected only by the standard consensus PCR assay and not by the PGMY-line blot. The signal obtained in the dot blot was weak for all seven samples, suggesting a low viral load. These seven samples scored positive on retesting with the PGMY-line blot. This could represent either variable amplification efficiency by PGMY primers or a low viral load. Due to the genetic diversity of HPV and the number of genital HPV types, the highest level of sensitivity for HPV detection can only be achieved by combining several different PCR assays (2, 19, 24, 28). However, this strategy is not practical for large-scale epidemiological studies and diagnostic purposes.

The PGMY primer system has shown better analytical sensitivity for several common genital HPV types, including those less efficiently amplified with MY09/MY11, such as types 35, 52, and 56 (4, 15, 28). In our study, samples positive for HPV exclusively with PGMY-line blot were found for each HPV genotype. The greater ability of PGMY-line blot to detect HPV types 45 and 52 over the MY system had also been reported in the first published description of PGMY primers (15). In our study, more samples contained HPV types identified exclusively by PGMY-line blot assay than exclusively by the standard consensus PCR assay. Considering typing results, the agreement between the two PCR assays was lower but still good. The increase in type-specific sensitivity of PGMY-line blot could be attributed to nonspecific or cross-reactivity of PGMY primers. However, most PGMY-positive, MY-negative samples were shown by repeat testing with both PCR assays and testing with GP5+/GP6+ primers to contain the HPV type detected initially by PGMY-line blot. Different results were obtained on these samples using MY09/MY11/HMB01 primers from different synthesis batches.

The increased sensitivity of PGMY-line blot could also be attributed to the use of Ampli *Taq* Gold DNA polymerase. However, Ampli *Taq* Gold was used for both PGMY and MY systems in the first published report on PGMY primers and increased sensitivity of PGMY-line blot was still demonstrated (15). We did not calculate the sensitivity and specificity of the PGMY-line blot for HPV typing since confirmatory testing was not performed on all samples. The purpose of the study was rather to evaluate the gain in diagnostic yield of the new PGMY system and how it could affect the rate of type-specific detection of HPV in clinical specimens.

The MY09/MY11/HMB01 primers had been shown previously to preferentially amplify some HPV types in specimens containing multiple types, generating inaccurate results (32, 33). PGMY-line blot improved the capacity of L1 consensus PCR to identify types implicated in multiple type infections, as reported previously (15). Discordant samples were more often those containing more than one type, illustrating the difficulty of using PCR assays to amplify and detect several types in the same reaction. Cycle sequencing has been reported to better detect some HPV types than the line blot assay, but it has not been found to perform well on samples containing several HPV types (33).

A limited number of experiments suggested that, as previously demonstrated, coamplification of HPV with β-globin reduces the level of sensitivity for HPV detection with consensus L1 primers (4, 33). The previous comparison of PGMY and MY09/11/HMB01 used β-globin coamplification and showed similar increases in HPV genotype detectability using PGMY, but the concentration of β-globin primers was smaller in the PGMY assay relative to the MY09/11HMB01 assay. This could have resulted in an improved amplification of HPV by reducing competition with β-globin coamplification. Since we did not use β-globin coamplification, the increase in PGMY amplification efficiency across the genotype spectrum appears to be independent of differences in coamplification profiles.

In conclusion, there was a good agreement between these two assays for HPV DNA detection and for HPV typing. We confirm the results obtained in the first evaluation of PGMY primers that demonstrated that this system is more sensitive than the standard consensus PCR assay, especially in samples containing multiple HPV types. Although detection of some HPV types was clearly improved, most HPV types were more frequently detected with the PGMY09/PGMY11 than MY09/MY11/HMB01 primers. Given its superior performance and the augmented probe spectrum offered by the PGMY-line blot test, its use should allow a better assessment of the natural history of HPV.

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REFERENCES

1. **Alani, R. M., and K. Munger.** 1998. Human papillomaviruses and associated malignancies. J. Clin. Oncol. **16:**330–337.
2. **Baay, M. F., W. G. Quint, J. Koudstaal, H. Hollema, J. M. Duk, M. P. Burger, E. Stolz, and P. Herbrink.** 1996. Comprehensive study of several general and type-specific primer pairs for detection of human papillomavirus

DNA by PCR in paraffin-embedded cervical carcinomas. J. Clin. Microbiol. **34:**745–747.
3. **Bauer, H. M., Y. Ting, C. E. Greer, J. C. Chambers, C. J. Tashiro, J. Chimera, A. Reingold, and M. M. Manos.** 1991. Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA **265:**472–477.
4. **Coutlee, F., P. Gravitt, H. Richardson, C. Hankins, E. Franco, N. Lapointe, H. Voyer, and the Canadian Women's HIV Study Group.** 1999. Nonisotopic detection and typing of human papillomavirus DNA in genital samples by the line blot assay. J. Clin. Microbiol. **37:**1852–1857.
5. **Coutlée, F., C. Hankins, N. Lapointe, and the Canadian Women's HIV Study Group.** 1997. Comparison between vaginal tampon and cervicovaginal lavage specimens collection for detection of human papillomavirus DNA by the polymerase chain reaction. J. Med. Virol. **51:**42–47.
6. **Coutlée, F., M. H. Mayrand, D. Provencher, and E. Franco.** 1997. The future of HPV testing in clinical laboratories and applied virology research. Clin. Diagn. Virol. **8:**123–141.
7. **Coutlée, F., A. M. Trottier, G. Ghattas, R. Leduc, E. Toma, G. Sanche, I. Rodrigues, B. Turmel, G. Allaire, and P. Ghadirian.** 1997. Risk factors for oral human papillomavirus in adults infected and not infected with human immunodeficiency virus. Sex. Transm. Dis. **24:**23–31.
8. **de Roda, H., J. M. Walboomers, A. J. van den Brule, C. J. Meijer, and P. J. Snijders.** 1995. The use of general primers GP5 and GP6 elongated at their 3′ ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J. Gen. Virol **76:**1057–1062.
9. **de Villiers, E.-M.** 1994. Human pathogenic papillomavirus types: an update. Curr. Opin. Microbiol. Immunol. **108:**2–9.
10. **Ellerbrock, T. V., M. A. Chiasson, T. J. Bush, X. W. Sun, D. Sawo, K. Brudney, and T. C. Wright.** 2000. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. JAMA **283:**1031–1037.
11. **Fleiss, J. L.** 1981. Statistical methods for rates and proportions. John Wiley and Sons Inc., New York, N.Y.
12. **Franco, E. L.** 1991. The sexually transmitted disease model for cervical cancer: incoherent epidemiologic findings and the role of misclassification of human papillomavirus infection. Epidemiology **2:**98–106.
13. **Franco, E. L., L. L. Villa, J. P. Sobrinho, J. M. Prado, M.-C. Rousseau, M. Désy, and T. E. Rohan.** 1999. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J. Infect. Dis. **180:**1415–1423.
14. **Franco, E. L. F.** 1996. Epidemiology of anogenital warts and cancer, p. 597–623. *In* R. Reid and A. T. Lorincz (ed.), Human papillomavirus I. W. B. Saunders, Philadelphia, Pa.
15. **Gravitt, P., C. L. Peyton, T. Q. Alessi, C. Wheeler, F. Coutlée, A. Hildesheim, M. Schiffman, D. R. Scott, and R. J. Apple.** 2000. Improved amplification of genital human papillomaviruses. J. Clin. Microbiol. **38:**357–361.
16. **Gravitt, P., C. L. Peyton, R. J. Apple, and C. Wheeler.** 1998. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by single-hybridization, reverse line blot detection method. J. Clin. Microbiol. **36:**3020–3027.
17. **Guerrero, E., R. W. Daniel, F. X. Bosch, X. Castellsagué, N. Munoz, M. Gili, P. Viladu, C. Navarro, M. L. Zubiri, N. Ascunce, L. C. Gonzales, L. Tafur, I. Izaraugaza, and K. V. Shah.** 1992. Comparison of Virapap, Southern blot hybridization, and polymerase chain reaction methods for human papillomavirus identification in an epidemiological investigation of cervical cancer. J. Clin. Microbiol. **30:**2951–2959.
18. **Hankins, C., F. Coutlee, N. Lapointe, P. Simard, T. Tran, J. Samson, L. Hum, and the Canadian Women's HIV Study Group.** 1999. Prevalence of risk factors associated with human papillomavirus infection in women living with HIV. Can. Med. Assoc. J. **160:**185–191.
19. **Harnish, D. G., L. M. Belland, E. E. Scheid, and T. E. Rohan.** 1900. Evaluation of human papillomavirus consensus primers for HPV detection by the polymerase chain reaction. Mol. Cell. Probes **13:**9–21.
20. **Hildesheim, A., M. H. Schiffman, P. E. Gravitt, A. G. Glass, C. E. Greer, T. Zhang, D. R. Scott, B. B. Rush, P. Lawler, M. E. Sherman, R. J. Kurman, and M. M. Manos.** 1994. Persistence of type-specific human papillomavirus infection among cytologically normal women. J. Infect. Dis. **169:**235–240.
21. **Ho, G. Y. F., R. Bierman, L. Beardsley, C. J. Chang, and R. D. Burk.** 1998. Natural history of cervicovaginal papillomavirus infection in young women. N. Engl. J. Med. **338:**423–428.
22. **Jacobs, M. V., H. de Roda, A. J. van den Brule, P. J. Snijders, C. J. Meijer, and J. M. Walboomers.** 1995. Group-specific differentiation between high- and low-risk human papillomavirus genotypes by general primer-mediated PCR and two cocktails of oligonucleotide probes. J. Clin. Microbiol. **33:**901–905.
23. **Jacobs, M. V., P. J. F. Snidjers, A. J. C. van den Brule, T. J. M. Helmerhorst, C. Meijer, and J. Walboomers.** 1997. A general primer GP5+/GP6+-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. J. Clin. Microbiol. **35:**791–795.
24. **Kleter, B., L.-J. van Doorn, L. Schrauwen, A. Molijn, S. Sastrowijoto, J. ter Schegget, J. Lindeman, B. ter Harmsel, M. Burger, and W. Quint.** 1999. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J. Clin. Microbiol. **37:**2508–2517.
25. **Manos, M. M., Y. Ting, D. K. Wright, A. J. Lewis, T. Broker, and S. M. Wolinski.** 1989. Use of the polymerase chain reaction amplification for the detection of genital human papillomaviruses. Cancer Cells **7:**209–215.
26. **Mayrand, M. H., F. Coutlée, C. Hankins, N. Lapointe, P. Forest, M. de Ladurantaye, the Canadian Women's HIV Study Group, and M. Roger.** 2000. Consecutive detection of HPV-16 DNA in genital samples does not always represent persistent infection as determined by single-strand conformation polymorphism and PCR sequencing analyses. J. Clin. Microbiol. **38:**3388–3393.
27. **Nobbenhuis, M. A., J. M. Walboomers, T. J. Helmerhorst, L. Rozendaal, A. J. Remmink, E. K. Risse, H. C. van der Linden, F. J. Voorhorst, P. Kenemans, and C. J. Meijer.** 1999. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. Lancet **354:**20–25.
28. **Qu, W., G. Jiang, Y. Cruz, C. J. Chang, G. Y. Ho, R. S. Klein, and R. D. Burk.** 1997. PCR detection of human papillomavirus: comparison between MY09/MY11 and GP5+/GP6+ primer systems. J. Clin. Microbiol. **35:**1304–1310.
29. **Richardson, H., E. Franco, F. Coutlée, and P. Tellier.** 2000. Risk factors for incident cervical human papillomavirus infection in Montreal university students. 18th International Papillomavirus Conference, Barcelona, Spain.
30. **Schiffman, M. H., and L. A. Brinton.** 1995. The epidemiology of cervical carcinogenesis. Cancer **76**(Suppl.)**:**1888–1901.
31. **Tate, J. E., Y. C. Yang, J. Shen, C. M. McLachlin, E. E. Sheets, and C. P. Crum.** 1996. A comparison of early (E7) and late (L1) primer-mediated amplification of papillomaviral DNA in cervical neoplasia. Mol. Cell. Probes **10:**347–351.
32. **Tucker, R. A., P. R. Johnson, W. C. Reeves, and J. P. Icenogle.** 1993. Using the polymerase chain reaction to genotype human papillomavirus DNAs in samples containing multiple HPVs may produce inaccurate results. J. Virol. Methods **43:**321–333.
33. **Vernon, S. D., E. R. Unger, and D. Williams.** 2000. Comparison of human papillomavirus detection and typing by cycle sequencing, line blotting, and hybrid capture. J. Clin. Microbiol. **38:**651–655.
34. **Villa, L. L.** 1997. Human papillomaviruses and cervical cancer. Adv. Cancer Res. **71:**321–341.
35. **Walboomers, J. M. M., M. V. Jacobs, M. M. Manos, F. X. Bosch, J. A. Kummer, K. V. Shah, P. J. F. Snijders, J. Peto, C. J. L. M. Meijer, and N. Munoz.** 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J. Pathol. **189:**12–19.
36. **zur Hausen, H.** 1996. Papillomavirus infections–-a major cause of human cancers. Biochim. Biophys. Acta **1288:**F55–F78.